A POPULATION STUDY OF *EUGLENA GRACILIS* VAR. *UROPHORA* AS AFFECTED BY CERTAIN CARCINOGENIC AGENTS

by

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Dean of Graduate Division

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CHAPTER I

INTRODUCTION

Factors which influence growth in protozoans are numerous and include many chemical, physical, and environmental agents. In an attempt to explain the various actions resulting from the use of such agents in protozoan cultures, the control of many of the variables is necessary. The successful elimination or control of these variables will, of course, aid in the ease by which the results of the investigation may be interpreted.

Among the various chemical substances which may be capable of modifying a protozoan population are the carcinogens. It is of some interest to the biologist to determine the effects, resulting from the action of carcinogenic substances on phototrophic organisms.

In an attempt to determine the effects produced by the action of such chemical carcinogens on Euglena gracilis var. urophora, three substances which may have carcinogenic action were selected for investigation: Ethyl Carbamate (Urethane), a substance which will initiate lung cancer in mice, o-Toluidine, an agent capable of producing cancer of the bladder, and Beryllium Nitrate which in this form is unknown for ability to produce cancer, although the metal

Beryllium is believed capable of producing cancer of the lung.

In Euglena, any effect caused by the addition of carcinogens to a culture media, might possibly be noted through modification of cellular structure, or through changes occurring in the total population.

The purpose of this investigation is to: (1) determine if any change occurs in the population as a result of the addition of certain carcinogenic agents in varying concentrations in a bacteria-free environment of Euglena gracilis var. urophora, and (2) determine if varying concentrations of certain carcinogens produce a change in protoplasmic volume of Euglena gracilis var. urophora.

CHAPTER II

MATERIALS AND METHODS

The species of Euglena used in this investigation was Euglena gracilis var. urophora, obtained from Professor Provasoli at Haskin's Laboratory, New York. This aseptic culture of Euglena was grown in Provasoli medium which consisted of 0.1 per cent Sodium Acetate (Coleman and Bell, c.p.), 0.2 per cent Trypticase (Baltimore Biological) and 0.1 per cent Thiopeptone (Wilson and Co., Chicago), adjusted to a pH 6.0.

The carcinogens used in this investigation were selected on the basis of three properties: (1) they must be able to produce cancer in experimental animals, (2) they must be water soluble so as to be a direct environmental influence, (3) the Euglena must be able to grow in the culture media with the addition of the carcinogens. The carcinogens used were Ethyl Carbamate (Urethane), o-Toluidine, and Beryllium Nitrate. The various concentrations of the carcinogens were determined as follows. Serial dilutions of each of the three carcinogens above were prepared and tested for action against Euglena. The lowest concentration of each carcinogen that exhibited toxicity, i.e., caused death, of the Euglena was determined. One-half of the lowest toxic concentration of each carcinogen and decreasing concentrations from that point

were subsequently used for experimentation. The various carcinogens were added to the regular Provasoli culture media in the following concentrations: Urethane, 1 per cent, 0.1 per cent, 0.01 per cent, and 0.001 per cent; o-Toluidine, 0.05 per cent, 0.005 per cent, 0.0005 per cent, and 0.00005 per cent; Beryllium Nitrate, 0.05 per cent, 0.005 per cent, 0.0005 per cent, and 0.00005 per cent. Urethane and o-Toluidine were added directly to the Provasoli medium and autoclaved at fifteen pounds pressure for twenty minutes. Beryllium Nitrate solutions were prepared by dissolving the Beryllium Nitrate in distilled water in the proper concentrations, and adding this mixture to a regular culture media in a sterile room, after each had been autoclaved separately. The later procedure was to prevent the Beryllium Nitrate from precipitating out in solution when autoclaving.

All glassware used in these experiments were washed with Sulphuric Acid-Potassium Dichromate solution as suggested by Schoenborn (1949), rinsed five times in tap water and two times in distilled water and air dried. All culture media was adjusted to pH 6.0 using a Beckmann pH meter and all culture media was autoclaved for twenty minutes at fifteen pounds pressure.

Euglena gracilis var. urophora was grown in stock cultures, in 250 cc. pyrex Erlenmeyer flasks containing 150 cc. of Provasoli media, until heavy growth was apparent (approximately fourteen to eighteen days). One cc. of Euglena from this stock solution was transferred in a one cc. sterile

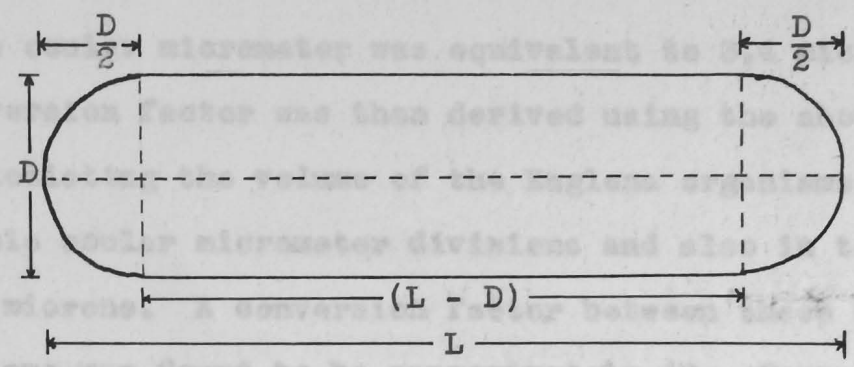
pipette to nine cc. of sterile culture media in test tubes (Kimble, screw cap type, 20 by 150 mm., 30 cc.). In each experiment, twenty-five test tubes were used as controls, and twenty-five test tubes for each concentration of carcinogen was used. The transfers from stock culture to the test tubes were carried out in a sterile room under ultra-violet light.

After inoculation of the test tubes with Euglena, the test tubes were placed in a water bath in test tube racks. No attempt was made to maintain a constant temperature in the water bath; however, the temperature during the summer months was approximately 24° to 25° C., and during the fall and winter months, the temperature of the water bath was approximately 22° to 23° C. Although the temperature was not constant, any change occurring in the organisms as a result of temperature fluctuation would be recorded in the control culture. The experiments were carried out under constant light, the source of which was two sixty watt electric light bulbs mounted directly above the water bath to give as uniform illumination as possible. Each experiment was carried out for a period of time of fourteen to eighteen days.

Each day a control test tube of Euglena and test tubes of each concentration of carcinogen were opened and the concentration of Euglena per cc. determined. A dilution method of determining the concentration of the population was employed. A pipette was calibrated and found to contain thirty-eight drops per cc. After thorough shaking, one drop

of Euglena from a culture tube was added to nineteen drops of killing solution (0.1 per cent Mercuric Chloride) in a 2.5 cc. vial. Five such samples were made from each culture tube including the controls. From each of the vials, after thorough shaking, one drop of this mixture was placed on each end of a microscope slide. On the underside of the counting slide was attached one mm. ruled graph paper to serve as guide lines for direct count of the number of Euglena per drop. Thus a total of ten one drop samples from the control and each concentration of carcinogen was made every twenty-four hours for fourteen to eighteen days. The population of Euglena per cc. is determined by multiplying the number of organisms counted by 760. The figure 760, was derived by multiplying the figure 20, the dilution of the Euglena organisms, times 38, the number of drops per cc. in the pipette. Euglena were counted using a compound microscope with a 10x ocular and a 5.1x objective, using an Adams laboratory hand counter (Clay-Adams Company).

A formula was derived to approximate the protoplasmic volume of the Euglena under consideration. The species of Euglena used in this investigation, Euglena gracilis var. urophora was found to closely resemble a cylinder with a hemisphere attached by the flat surface at each end of the cylinder. The formula for determining the volume of a cylinder can be given as $(L - D)(\pi)\left(\frac{D}{2}\right)^2$. The formula for determining the volume of a sphere, which would be the geometric figure when adding together the two hemispheres



on either end of the cylinder, can be given as $(\frac{4}{3})(\pi)(\frac{D}{2})^3$. Combining the volume formula of a cylinder algebraically with the volume formula for a sphere, an expression for determining the volume of the Euglena organism can be derived as follows. $V_E = V_C + V_S$ where V_E can be given as the volume of Euglena, V_C = volume of a cylinder, and V_S = volume of a sphere.

$$V_E = V_C + V_S$$

$$V_E = (L - D)(\pi)(\frac{D}{2})^2 + (\frac{4}{3})(\pi)(\frac{D}{2})^3$$

$$V_E = (L - D)(3)(\frac{D^2}{4}) + (\frac{4}{3})(3)(\frac{D^3}{8})$$

$$V_E = \frac{3LD^2 - D^3}{4}$$

Using the formula as derived above, only two measurements of the Euglena organisms are required, total length and diameter. Since measurements of the Euglena organisms were made in terms of microscopic ocular micrometer divisions, a constant must be determined to convert the above formula to terms of cubic microns. It was determined that one division

of the ocular micrometer was equivalent to 3.4 microns. A conversion factor was then derived using the above formula, by calculating the volume of the Euglena organisms in terms of cubic ocular micrometer divisions and also in terms of cubic microns. A conversion factor between these two expressions was found to be approximately 40. Hence the formula for calculating the volume of Euglena protoplasm, used in this investigation was $V_E = 40(\frac{3LD^2 - D^3}{4})$. Fifty organisms from the control and fifty organisms from each concentration of the carcinogens were measured every twenty-four hours for the length of the experiments, from fourteen to eighteen days. All measurements were made with a compound microscope using a 44x objective and a 10x ocular.

CHAPTER III

REVIEW OF THE LITERATURE

The effects produced by carcinogenic substances on protozoans have been reported by several investigators, the specific agent, protozoan, procedure and effects differing among different investigators.

Mottram (1940) working with Paramecium sp. reported that Benzpyrene, a strong carcinogenic agent produced abnormal forms when applied to the environment of these organisms. In 1944, Mottram, working with Colpidium sp. measured individuals and micronuclei of abnormal organisms produced by blastomatogenic agents and found that the majority were of varying multiple constitution. This same investigator (1944a) found that exposure of ciliates to a variety of blastogenic agents resulted in the production of abnormal races, the abnormal ciliates always beginning as double or multiple organisms, due to inhibition of fission or of both fission and mitosis.

Wolman (1940) investigated the effects of three carcinogenic hydrocarbons, 3,4 Benzpyrene, 1,2,5,6 Dibenzanthracene, and Methylcholanthrene on Paramecium sp. and found that the carcinogens produced a proliferative effect, each substance having an optimal concentration. This investigator

also tested four non-carcinogenic substances, 1,4 Diphenylanthracene, 9,10 Diphenylanthracene, 1,4,9,10 Tetraphenylanthracene, and Chrysene and found no effects in any concentration tested.

Spencer and Melroy (1940) found that the carcinogen Methylcholanthrene, generally stimulated the cell division of Paramecium multimicronucleatum, but found no evidence to suggest an increase in the period of survival of individual organisms. These investigators (1941) confirm the findings of Mottram when they report the presence of abnormal forms of Paramecia during early stages of experiments. In 1949, Spencer and Melroy found that exposure of Paramecium multimicronucleatum over a period of seven to nine years, increased the life span of the strain, the organisms living longer and reaching higher population levels than the non-methylcholanthrene-adapted Paramecia.

Investigating the effects produced by three carcinogens, Methylcholanthrene, Scharlach Red, and 3,4 Benzpyrene, on Paramecium caudatum, Tittler and Kobrin (1942) found that similar abnormalities in the form of swellings, vacuolizations of cytoplasm and blistering of the pellicle occurred with all substances, regardless of the different concentrations used. They failed to produce abnormal monster organisms as Mottram (1940) reported.

Sullivan (1948) working with Colpidium campylum reported that cholesterol, when added to a proteose-peptone media, delayed the maximum growth for one to two days as

compared with the controls, cholesterol exerting a slight stimulating effect on the growth of these organisms.

Tittler (1948) used three carcinogens, Methylcholanthrene, 1,2,5,6 Dibenzanthracene, and 3,4 Benzpyrene and attempted to determine the effects on Tetrahymena geleii. He used four methods of measuring growth, cell counts, optimal density, dry weight, and nitrogen content, and found that none of the carcinogens used affected growth.

Investigating the effect of malignant human serum on Paramecium caudatum, Egan (1950) reports that there is an increase in number of organisms in non-malignant serum over normal serum, while there is a slight increase in number of organisms in malignant tumor serum, but below that of normal serum. This work was reported as a toxicity test using Paramecia for the diagnosis of cancer.

An investigation approaching carcinogens in terms of action on chlorophyll-bearing organisms, was the work of Elliott (1938) who investigated the action of certain plant hormones on Euglena gracilis, Khawkinea halli, and Colpidium striatum. Using β-3-n Indolebutyric acid, γ-3 Indolepropionic acid, and 3-Indoleacetic acid (Heteroauxin), Elliott determined that these compounds produced a marked acceleration of growth of Euglena gracilis, whereas the indol compounds did not cause acceleration of growth of Khawkinea halli or Colpidium striatum at any pH tested. Thus the accelerating effect of these phytohormones appears to be in its relationship to the presence of chlorophyll.

CHAPTER IV

RESULTS AND INTERPRETATION OF DATA

The results of the investigation of the effects of certain carcinogens on Euglena gracilis var. urophora are shown in the accompanying illustrations. The population is recorded as the mean of ten samples from each culture, and the protoplasmic volume is given as the total volume of fifty Euglena organisms.

The experiment showing the effect of o-Toluidine on this species of Euglena are illustrated in Figure 1 and Figure 2.

Figure 1 shows the effect of o-Toluidine on the population of Euglena. The initial concentration of Euglena at the beginning of the experiment was approximately 13,000 per cc. The end of the logarithmic growth phase was reached at the end of four days, the concentration in the control reaching 125,000 per cc. Following the logarithmic phase was an equilibrium period, in which the concentration of Euglena per cc. fluctuated between 128,000 and 169,000 per cc. The four concentrations of o-Toluidine used in the experiment was 0.05 per cent, 0.005 per cent, 0.0005 per cent, and 0.00005 per cent. The end of the period of logarithmic growth for the experimental cultures of Euglena was the same as the control,

four days, the cultures varying in concentration from about 81,000 per cc. in the 0.05 per cent concentration to 85,000 per cc. in the 0.00005 per cent concentration. The concentration of Euglena in the remaining days of the investigation varied generally between 88,000 per cc. and 131,000 per cc. with a few fluctuations above the higher extreme. In general, the concentration of Euglena per cc. in all four concentrations of o-Toluidine, remained below that of the control, with the two highest concentrations of o-Toluidine, 0.05 per cent and 0.005 per cent having the lowest number of Euglena organisms per cc. respectively. The third highest concentration of o-Toluidine, 0.0005 per cent appears to reach the highest number of Euglena organisms per cc., but still remaining below that of the control.

Figure 2 is an illustration showing the effects of o-Toluidine in terms of effect on protoplasmic volume of these organisms, as determined by the formula derived in an earlier chapter. The total volume of fifty Euglena organisms at the beginning of the experiment was 1.43 million cubic microns. On the first day, the total volume of the control culture increased to 1.84 million cubic microns which was the greatest volume of the control culture during the experiment. On the third day, the volume of the control decreases to 1.47 million cubic microns, rising to 1.63 million cubic microns, then a gradual decrease in protoplasmic volume, falling to 1.25 million cubic microns on day ten. The 0.00005 per cent culture of Euglena appears to follow a series

of peaks and declines in protoplasmic volume. On day two, the total protoplasmic volume was 1.87 million cubic microns, falling to 1.43 million cubic microns on day four, rising to 1.85 million cubic microns on day six, falling to 1.38 million cubic microns on day seven. The cycle is continued, reaching peaks of 1.65 million cubic microns on day eight, 1.53 on day ten, and 1.56 million cubic microns on day fourteen, and lowest depressions on days nine, and twelve, with a volume of 1.37 and 1.40 million cubic microns respectively. The culture of Euglena organisms in the 0.05 per cent concentration of o-Toluidine reaches a total volume of 2.26 million cubic microns on day one, the greatest protoplasmic volume in the experiment, falling to 1.40 million cubic microns on day sixteen, then reaching on equilibrium period, fluctuating between 1.36 and 1.49 million cubic microns. The other concentrations of o-Toluidine, 0.005 per cent and 0.0005 per cent, follow the same general pattern, with some fluctuating above and some below the control except during day eight to day twelve, when the protoplasmic volume of the control is lower than all concentrations of this carcinogen.

Figure 3 shows the effect of Beryllium Nitrate on the population of Euglena. The initial concentration of Euglena per cc. at the beginning of the experiment was approximately 25,000. Four concentrations of Beryllium Nitrate were used, 0.05 per cent, 0.005 per cent, 0.0005 per cent, and 0.00005 per cent. The end of the logarithmic growth phase of all cultures was reached at the end of six days; the

concentration at that time was about 120,000 per cc. During the remainder of the experiment which was carried out for seventeen days, each of the cultures of Euglena in all concentrations of Beryllium Nitrate appear to fluctuate around the control varying in concentration per cc. between 135,000 and 145,000. It may be pointed out that the Euglena culture in 0.005 per cent of Beryllium Nitrate consistently appears below that of the control, whereas the smallest concentration of Beryllium Nitrate, 0.00005 per cent, consistently appears above the control.

Figure 4 is an illustration showing the total protoplasmic volume of fifty Euglena organisms as affected by Beryllium Nitrate. The volume of Euglena at the beginning of the experiment was 1.73 million cubic microns. The control culture on day one increases in volume to 2.33 million cubic microns which was the greatest volume reached by the control culture. The control then declines in volume to 1.56 million cubic microns on day six, then forming a series of peaks on days seven, nine, and twelve, with volumes of 1.86, 1.97, and 2.08 million cubic microns respectively. Following day twelve, the protoplasmic volume declines for the remaining five days of the experiment with a volume of 1.79 million cubic microns on day seventeen. The various concentrations of Beryllium Nitrate follow the control culture in its general form except in a few cases. The highest concentration of Beryllium Nitrate used, 0.05 per cent, shows three deviations from the control, declining to 1.20 million cubic microns on day

one, reaching 3.23 million cubic microns on day four, and falling to 1.38 million cubic microns on day six; the other days in the experiment followed the graph of the control very closely. It may be pointed out that during the first three days of the experiment, the control culture protoplasmic volume was above all concentrations of the Beryllium Nitrate cultures, although the amount of variance in most cases was slight.

Figure 5 is a graph showing the effects produced by Urethane on the population of Euglena. The initial concentration of all cultures at the beginning of the experiment was approximately 11,000 organisms per cc. The end of the logarithmic growth phase of the control was on day five, the population at that time reaching 117,000 Euglena per cc. The control culture of Euglena rises to a concentration of 141,000 per cc. on day seven, then maintaining an equilibrium phase with the concentration of organisms fluctuating between 126,000 and 146,000 Euglena per cc. up to day fourteen. On day sixteen, the population of Euglena had declined to 120,000 organisms per cc., increasing to 134,000 organisms per cc. on day eighteen. Four concentrations of Urethane were used in the experiment, 1 per cent, 0.1 per cent, 0.01 per cent and 0.001 per cent. The population of Euglena organisms in three of the concentrations of this carcinogen, 0.1 per cent, 0.01 per cent, and 0.001 per cent follow the control culture very closely, with only minor variance. It may be pointed out that the population of these three cultures containing Urethane were

all below that of the control during the logarithmic growth phase. The population in the 0.001 per cent culture of organisms was usually found to be above the control after day eight, except on days twelve, fourteen and seventeen, when a slight decline below the control was evident. On day seventeen, these three cultures of Euglena were all below the control, but maintaining a greater population level on the two days preceeding and the day following day seventeen. The population of Euglena in the 1 per cent culture of Urethane shows a great variance from the control and other three cultures containing Urethane. Beginning with day one, the population of Euglena was 6500 per cc. rising to 8900 per cc. on day two, then beginning a gradual decline to concentration of organisms to 2900 per cc. on day six. The population rises slightly on days seven and eight and reaches the lowest population level of the experiment on day nine, 2500 organisms per cc. Beginning with day ten, the population of organisms increases, varying from 8500 to 20,000 Euglena per cc. between days ten and sixteen. The population of Euglena declines to 10,000 per cc. on day seventeen and increases to 13,000 per cc. on day eighteen.

The relationship between the various concentrations of Urethane and the protoplasmic volume of Euglena are illustrated in figure 6. The volume of Euglena protoplasm of fifty organisms at the beginning of the experiment was 1.35 million cubic microns. The control culture of Euglena increases to the highest volume on day two, reaching 2.47 million cubic

microns, declining to a volume of 1.52 million cubic microns on day four. From this time, the total protoplasmic volume of the control varies between 1.40 and 1.89 million cubic microns, reaching peaks on day nine and day fourteen, and depressions on day seven and day twelve. Three of the concentrations of Urethane, 0.1 per cent, 0.01 per cent, and 0.001 per cent follow the same general form as the control with possibly two exceptions. On day two, the 0.01 per cent Urethane culture increases to a total volume of 2.92 million cubic microns, then following the control. The smallest concentrations of Urethane, 0.001 per cent follows the control graph to day sixteen, where the total volume increased to 2.65 million cubic microns. As in the population curve, the culture of organisms in 1 per cent Urethane showed the most marked deviation from the control culture. On day two, the protoplasmic volume of this culture reaches 2.14 million cubic microns, declines to 2.65 million cubic microns on day four, and rises to 3.92 million cubic microns on day seven. Following this point, the protoplasmic volume begins a decline with a series of minor peaks on days ten, fourteen and sixteen, with total protoplasmic volumes of 3.06, 2.72, and 2.52 million cubic microns respectively. On day eighteen, the final day of this experiment, the total volume had declined to 2.21 million cubic microns.

Figure 7 is an illustration attempting to show a relationship between figures 5 and 6. In this graph, combining the average protoplasmic volume with population figures

attained in the experiment with Urethane, the illustration shows total protoplasmic volume per total population. At the beginning of the investigation, the protoplasmic volume per total population of organisms was 318 million cubic microns. From this point, the total protoplasmic volume of the control is increased to 4.13 billion cubic microns at the end of day six. From this point the protoplasmic volume fluctuates generally between 3.97 and 5.04 billion cubic microns, reaching peaks on day ten and day fourteen, with 4.97 and 5.04 billion cubic microns respectively. Three of the concentrations of Urethane, 0.1 per cent, 0.01 per cent and 0.001 per cent follow the same general form as the control except for one day in the 0.001 per cent concentration of Urethane. On day sixteen, the total protoplasmic volume increases to 8.08 billion cubic microns, then decreases markedly on day seventeen to 3.67 billion cubic microns. The graph of the 1 per cent Urethane culture follows the same form as the population curve in figure 5. The total protoplasmic volume per population rises to 495 million cubic microns on day three, with a gradual decline to day six, a slight increase in volume on day seven, declining to a volume of 123 million cubic microns on day nine. Beginning with day ten, the protoplasmic volume increases to 582 million cubic microns and rises gradually to a volume of 1.05 billion cubic microns on day sixteen and declining to 592 million cubic microns on day eighteen.

Figure 8 is a diagram representing an experiment

showing the effects of Urethane on a population of Euglena. Two controls, two sets of cultures of 0.001 per cent Urethane, and one culture, an intermediate between two concentrations, 1 per cent and 0.1 per cent, used in figure 5, a culture containing 0.5 per cent Urethane were investigated. Beginning with a population level of 14,000 Euglena per cc. at the beginning of the experiment, the control cultures reach the end of the logarithmic growth phase at the end of six or seven days with a concentration of about 185,000 organisms per cc. From this point, the population of Euglena organisms declines and reaches an equilibrium phase varying generally between 136,000 and 156,000 organisms per cc. The other cultures containing Urethane follow the same general growth curve as the control, but usually have a smaller population of organisms per cc.

PLATE I

EFFECT OF o-TOLUILINE AND BERYLLIUM NITRATE
ON POPULATION AND PROTOPLASMIC VOLUME
OF EUGLENA GRACILIS VAR. UROPHORA

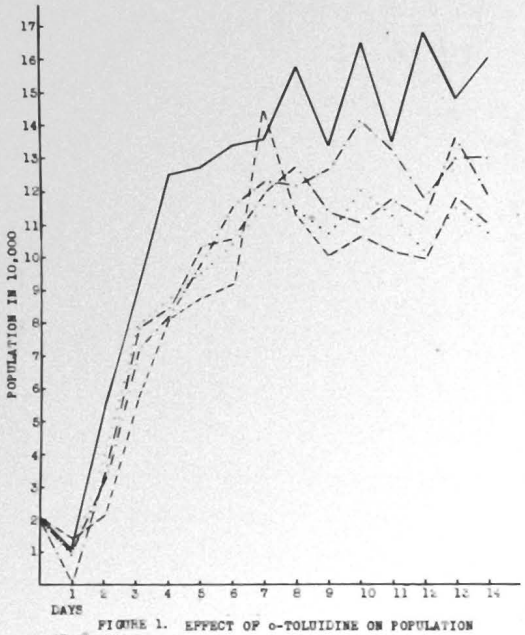


FIGURE 1. EFFECT OF o-TOLUIDINE ON POPULATION OF EUGLENA.

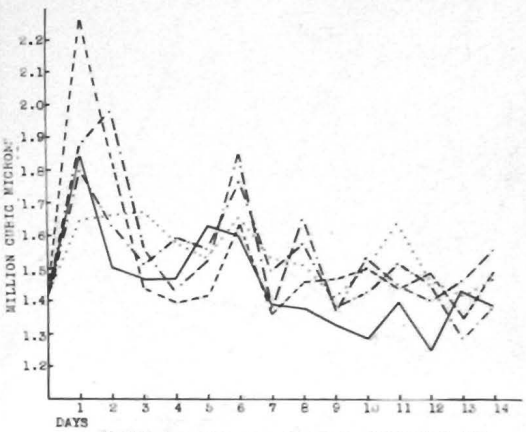


FIGURE 2. EFFECT OF o-TOLUILINE ON PROTOPLASMIC VOLUME OF EUGLENA (Sum of volumes of fifty organisms).

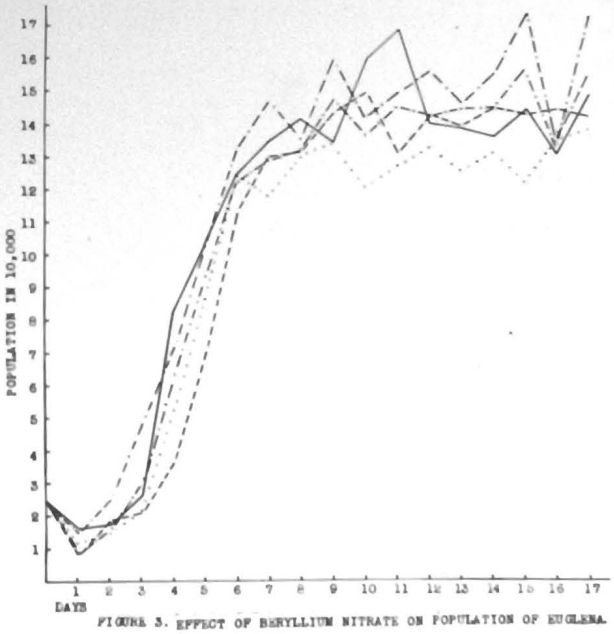


FIGURE 3. EFFECT OF BERYLLIUM NITRATE ON POPULATION OF EUGLENA.

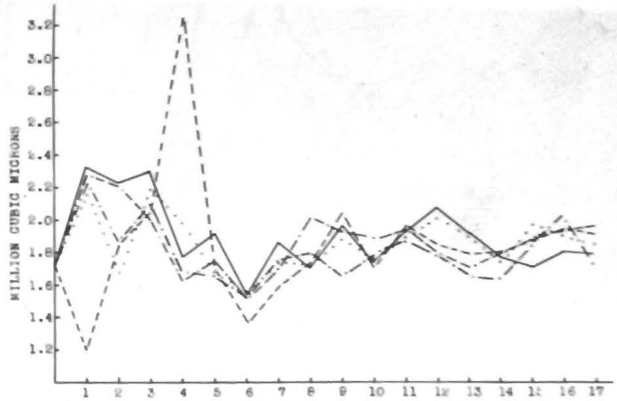


FIGURE 4. EFFECT OF BERYLLIUM NITRATE ON PROTOPLASMIC VOLUME OF EUGLENA (Sum of volumes of fifty organisms).

LEGEND

CONTROL
0.05 PER CENT
0.005 PER CENT
0.0005 PER CENT
0.00005 PER CENT

PLATE II

EFFECT OF URETHANE ON POPULATION AND PROTOPLASMIC VOLUME OF EUGLENA GRACILIS VAR. URAPHORA

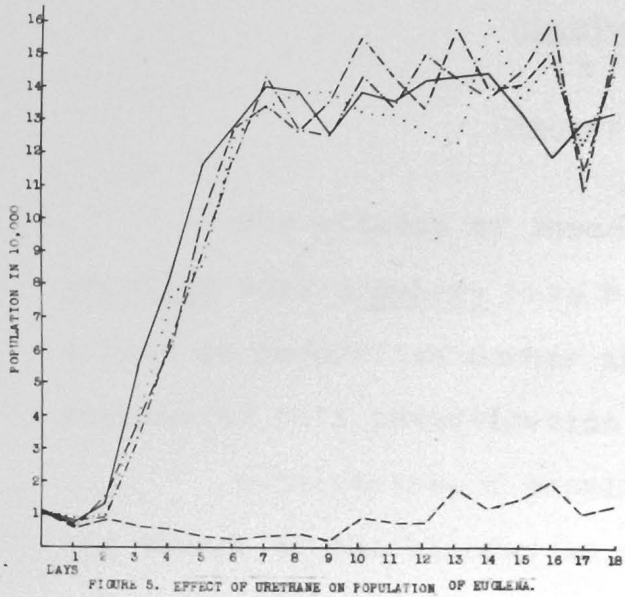


FIGURE 5. EFFECT OF URETHANE ON POPULATION OF EUGLENA.

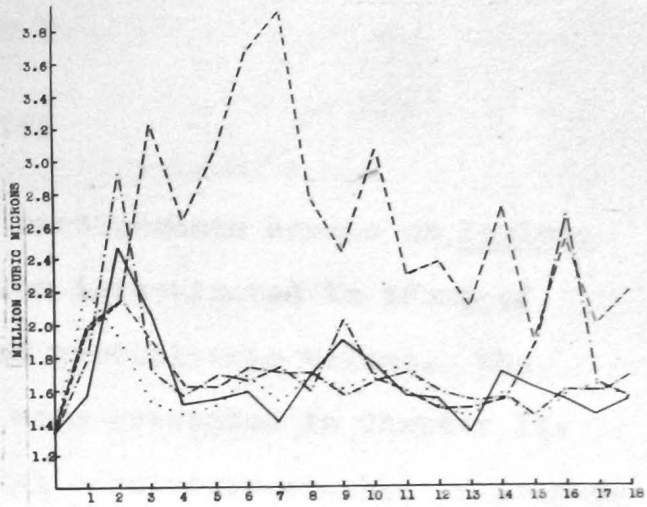


FIGURE 6. EFFECT OF URETHANE ON PROTOPLASMIC VOLUME OF EUGLENA (Sum of volumes of fifty organisms)

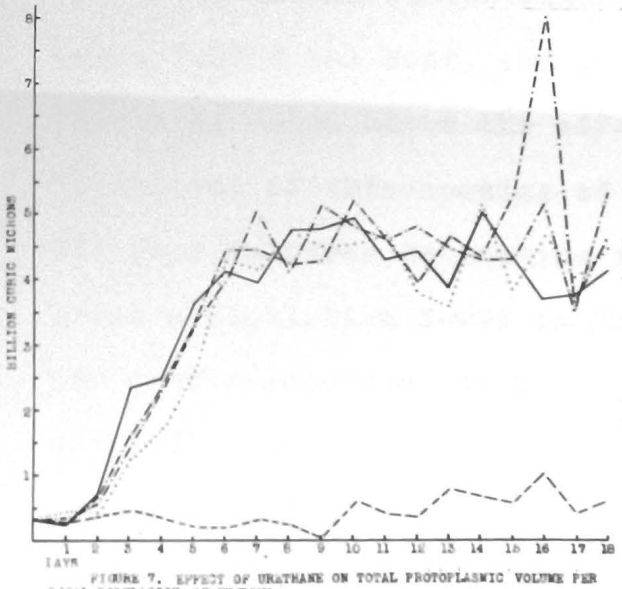


FIGURE 7. EFFECT OF URETHANE ON TOTAL PROTOPLASMIC VOLUME PER TOTAL POPULATION OF EUGLENA.

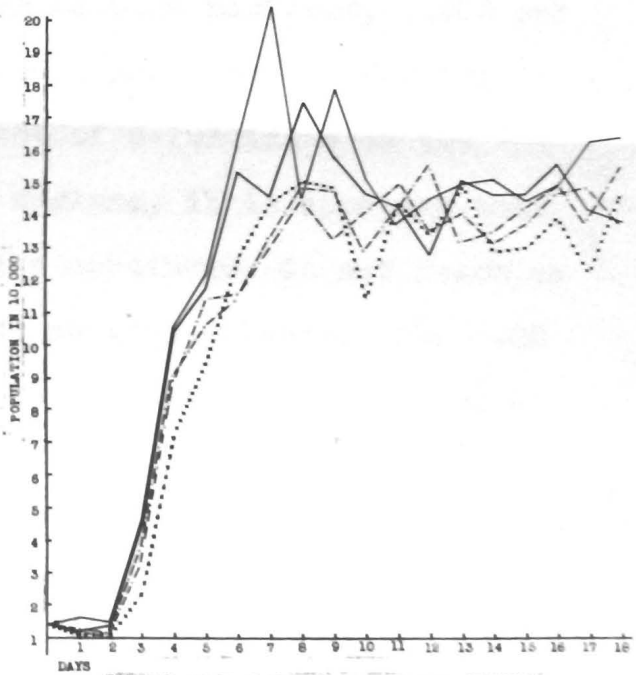


FIGURE 8. EFFECT OF URETHANE ON POPULATION OF EUGLENA.

LEGEND

——— CONTROL
– – – – 1.0 PER CENT
• • • • 0.5 PER CENT
. . . . . 0.1 PER CENT
— · — · 0.01 PER CENT
— — — 0.001 PER CENT

CHAPTER V

DISCUSSION

The effects of three carcinogenic agents on Euglena gracilis var. urophora have been investigated in terms of effect on population number and protoplasmic volume. The results of this investigation were presented in Chapter IV.

o-Toluidine, a chemical carcinogen capable of producing cancer of the bladder in mice, was added to the Provasoli culture media in concentrations of 0.05 per cent, 0.005 per cent, 0.0005 per cent, and 0.00005 per cent. Referring to Figure 1, which shows the effect of o-Toluidine on the population of this species of Euglena, it is apparent that all four cultures containing the carcinogen do not reach as great a population level as the control culture. The 0.05 per cent o-Toluidine culture, the highest concentration of chemical used, has generally the lowest population level, whereas the 0.0005 per cent culture has generally the highest population level of any of the experimental cultures. From these data, it seems apparent that o-Toluidine has a slight inhibitory action on the population of this species of Euglena, the effects increasing with the concentration of carcinogen.

The effects of o-Toluidine on the protoplasmic volume of Euglena are shown in Figure 2. The graph shows that the

protoplasmic volume of Euglena organisms, with exceptions of day two through five, is greater for the cultures of Euglena containing o-Toluidine than for the control culture. This would seem to indicate that the Euglena organisms increase in size as the population of the organisms is slightly inhibited. The effect of o-Toluidine on this species of Euglena would tend to agree with Mottram (1944a) in that there probably occurred an inhibition of fission, when this chemical was placed in the culture media containing this species of Euglena.

Although the metal Beryllium is believed capable of producing lung cancer, the chemical compound Beryllium Nitrate is unknown as a carcinogen. This substance was used in this investigation because of interest in the compound even though it is questionable whether the substance meets the requirements as set up for selection of these carcinogens. At various time intervals, the number of organisms in different concentrations of the Beryllium Nitrate appears to increase, but it does not appear that any effect is produced by the action of this chemical as measured by comparison with the control. Fluctuations above and below the control occurs, but these appear to be only minor and do not form a pattern of different action.

Figure 4 shows the effects of Beryllium Nitrate in terms of protoplasmic volume. Two noticeable deviations from the control occurs in this illustration. If this chemical had produced noticeable effects on the protoplasmic volume

of these organisms, it seems likely that the effects would continue for more than two unrelated isolated points. The explanation of the cause of these marked deviations from the control culture might be explained using some factor other than the effects produced by this chemical. It must be concluded from the results of this investigation that Beryllium Nitrate does not alter protoplasmic volume of this species of Euglena in any concentrations used under the conditions set up in this investigation.

The effects of Urethane on the population and protoplasmic volume of Euglena gracilis var. urophora are illustrated in Figures 5 and 6. With the exception of the 1 per cent culture of Urethane, the effects of other concentrations of this chemical on population and volume, as compared with the control, seems negligible. This agrees with the findings of Tittler (1948) who reported that none of the carcinogens used affected growth of Tetrahymena geleii. The 1 per cent Urethane culture shows a marked inhibition of the population of Euglena. Following day two, this culture never approaches the control in number of organisms throughout the remainder of the experiment. The effect of this carcinogen on protoplasmic volume, as seen in Figure 6, shows again a noticeable difference in the 1 per cent culture of Urethane. In this culture, the protoplasmic volume of Euglena organisms is much greater than the control and other concentrations of Urethane. It is to be pointed out that the smallest population level is reached on day nine, whereas

the greatest protoplasmic volume occurs on day seven. In the 1 per cent Urethane culture, the Euglena organisms appeared many times unactive in clusters, becoming ovoid to spherical in appearance. No abnormalities in the form of swellings or blistering of the pellicle as reported by Tittler and Kobrin (1942) in Paramecium caudatum were observed.

Figure 7 shows the total protoplasmic volume per total population per cc. of Euglena organisms, as affected by Urethane. It is the 1 per cent culture that shows a significant difference from the control culture. Actually in terms of total protoplasmic volume per total population, the organisms in the 1 per cent Urethane culture do not show as great a difference from the control as when considered separately. The effect of one additional concentration of Urethane, 0.5 per cent is shown in Figure 8. In this experiment, this culture of Euglena, as in all others except the 1 per cent Urethane culture, does not alter significantly from the control.

In attempting to explain the action of this 1 per cent Urethane culture, it seems apparent that an inhibition of fission has occurred with an accompanying increase in volume of these organisms. It is suggested that this action may result from interference with Thymonucleic Acid synthesis since it is believed by some (Sinclair, 1950) that Urethane inhibits synthesis of this compound. It must be remembered that Urethane has been used as an anaesthetic

and interference with respiratory mechanisms may be responsible for this action.

It is suggested that cultures of Euglena be maintained for a longer period of time than carried out in this experiment and effects noted. Spencer and Melroy (1949) found that the length of time to which they applied the carcinogen Methylcholanthrene to Paramecium multimicronucleatum altered their results and such may be the action with Urethane on this species of Euglena.

CHAPTER VI

SUMMARY

1. Investigation of the effects of three carcinogenic substances, o-Toluidine, Beryllium Nitrate, and Urethane (Ethyl Carbamate) on Euglena gracilis var. urophora is reported.

2. Growth of Euglena organisms is measured in terms of population number, as determined by a dilution method of counting and protoplasmic volume as determined by the formula:

$$V_E = 40\left(\frac{3LD^2 - D^3}{4}\right).$$

3. The effects of o-Toluidine in concentrations of 0.05 per cent, 0.005 per cent, 0.0005 per cent, and 0.00005 per cent show a slight inhibitory action on the population with an accompanying increase in protoplasmic volume, the effects increasing with the concentration.

4. Beryllium Nitrate in concentrations of 0.05 per cent, 0.005 per cent, 0.0005 per cent, and 0.00005 per cent shows no significant difference in terms of effects on population and volume of this species of Euglena as compared with the control culture.

5. Urethane in concentrations of 0.1 per cent, 0.01 per cent and 0.001 per cent produces no change in population or volume. The 1 per cent culture of Euglena shows a marked inhibition of population with an accompanying increase in protoplasmic volume.

6. It is suggested that the action of 1 per cent Urethane on this species of Euglena is produced through inhibition of fission, possibly through failure of Thymonucleic Acid synthesis or interference with respiratory mechanisms.

7. It is suggested that Urethane-adapted cultures of Euglena be maintained over a longer period of time than recorded in this investigation to note possible effects which may occur.

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